

X-gal Staining Protocol

Solutions:

40x X-gal stock:

40mg/ml X-gal (-20Fz) in DMSO

-Store at -20°C

Reaction Buffer:

20mM potassium ferricyanide (K_3Fe) = 8.45g/L

20mM potassium ferrocyanide (K_4Fe) = 6.58 g/L

2mM $MgCl_2$ (= 2:1000 from 1 M stock)

in PBS pH 7.8 (check pH!!!)

-Store at 4°C

Final X-gal Solution:

Dilute X-gal stock 1/40 in prewarmed (37°C) reaction buffer (prepare before staining)

X-Gal staining of cryosections:

- Cut Cryosections
- Air Dry slides
- Fix in 0.5% glutaraldehyde in PBS for 10min at 4°C
(do not use paraformaldehyde)
- Wash in PBS 3X 5 min
- Stain in final X-gal solution at 37°C until color appears (0.5-24hrs usually over night)
- Wash in PBS
- Counter-stain with Nuclear Fast Red
- Dehydrate and Cover-slip